Integrated Protection in Oak Forests
IOBC-WPRS Bulletin Vol. 101, 2014
pp. 31-38

Pedologic characteristics and fungi community in unmanaged cork oak forest soil of two Mediterranean regions: Sardinia and Tunisia

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Abstract: The soil of unmanaged cork oak forests located both in Sardinia and in Tunisia was characterized. Soil samples were collected in both areas at the depth of 0-10 cm, to determine the pedologic characteristics [humidity, pH, total organic carbon (TOC), total nitrogen (N) and texture] and the fungi community. The data were tested for significance with analysis of variance (ANOVA) techniques. The soils of the two studied areas were significantly different as far as pH, TOC % and Sand content are concerned. The texture of Sardinia soil was mainly classified as "sandy-loam" and the Tunisian ones as "sandy-clay-loam". Concerning the fungi community in Sardinia soil, the most frequent fungi genera were *Trichoderma, Penicillium* and *Paecilomyces*. In Tunisian soil the dominant genus was *Penicillium* followed by the genus *Aspergillus*.

Key words: cork oak forest, Tunisia, Sardinia, pedologic characteristics, soil fungi

Introduction

Cork oak (*Quercus suber* L.) forests are dispersed in the entire Mediterranean Basin and they are associated with a remarkable biodiversity, constituting one of the best examples of sustainable forest environment and a unique ecosystem recognized for their ecological value. The economic role of the cork oak forests represents a sustainable balance between human activities and natural resources, and engenders direct benefits derived from a number of different activities carried on under cork canopies (Pereira, 2007; Silva & Catry, 2006). The cork oak forest supplies a diversity of non-timber forest products (NTFPs), as grazing, firewood, cork, and honey, playing a key role in the national economy in less favorable Mediterranean areas (Croitoru, 2007). For Tunisia grazing is an important benefit, and cork is the second most important NTFPs: in this region, with ca. 9.000 tons of cork produced per year. In Sardinia, 18.000 tons of raw material are produced every year, and the economic importance of cork oak forests is principally associated with the production of stoppers (Barberis *et al*., 2003). The management of cork oak forests in the Mediterranean basin has been mainly oriented towards maximizing cork production, and shrub cleaning is a common silvicultural practice. However, this practice may interfere with the conservation of biodiversity, because the area devoted to unmanaged forest preserves the typical composition of the species found in the understorey of old-growth forests (Perez-Ramos *et al.*, 2008). This fact has led to the increasing interest in the study of unmanaged cork oak forests. In the last years, Sardinia and Tunisia cork oak forests have been undergone to some investigations as an

important part of the Mediterranean regions ecosystem. A cooperation research project (Serra *et al*., 2005) highlighted that anthropic activities play a main role in the degradation of cork oak stands areas. The study of chloroplast DNA of *Quercus suber* populations of the western Mediterranean regions allowed to identify five haplotypes; the same haplotypes were shared by Sardinia and Tunisia suggesting that cork oak populations have persisted there for a very long time span without detectable DNA modifications (Magri *et al.*, 2007). However, in these regions the soil physical and chemical properties which are indicators of forest quality are still largely unknown. Fungal community is another important indicator of forest quality, being a primary component of its biodiversity. Fungi perform a key role on plant health, growth and activity as well as on the other biotic components of the ecosystem. Studies have been carried out on biodiversity of fungi inhabiting soil forest (Christensen, 1981; Kara & Asan, 2007; De Bellis *et al.*, 2007) but none expressly focused on the diversity or dynamics of micro-fungal population in the top soil of the unmanaged cork oak forest. A recent cooperation work was carried out among Mediterranean countries to increase the knowledge of the natural resources within an international research project (Nato ESP.MD.SFPP 981674), where the attention was focused on cork oak forest soils from Sardinia and Tunisia. This work aims to present some results obtained within the project: i.e the pedologic characteristics and the fungi community of the unmanaged cork oak forests soils in Sardinia (Tempio Pausania District) and in Tunisia (Tabarka region).

Material and methods

Study area and forest sites

The Sardinia area was within the forest of Cusseddu-Miali-Parapinta (67 ha surface, 450-470 m a.s.l.), located in the Northern part of the island in the District of Tempio Pausania, and certified since 2005 according to the Forest Stewardship Council Standards (SA FM/COC-001436). A stand of about 1 ha was selected (40° 54’ 48” N, 9° 08’ 00” E; 452 m a.s.l.), not managed since about 50 years, and characterized by the presence of *Fraxinus ornus* L., with elements of *Quercus suber* L. and *Q. pubescens* L. Willd.; the high density understorey is almost entirely composed of *Ruscus aculeatus* L., *Hedera helix* L. and *Smilax aspera* L. The Tunisia area was located in the North-Western, in the Tabarka district, within the Ras Rajel forest (36° 57’ 15” N, 8° 51’ 48” E, 55 m a.s.l.). This was a natural forest characterized by elements of *Q. suber* mixed with *Quercus canariensis, Pinus pinaster, Pinus pinea, Pinus radiata, Pinus brutia, Eucalyptus* spp. A maquis of dishomogeneous density with elements of *Erica arborea* and *Myrtus communis* was also present.

Soil samples collection

The soil was sampled in the period from February, 24 to March,6 of 2009 (average temperature in the period February-March 2009: 4-17 °C for Sardinia and 4-23 °C for Tunisia), following the international standards (ISO 10381-1: 2002). Within each area three locations were randomly selected; from each location a composite sample was collected from 5 sub-samples (from 0 to 10 cm depths) and sieved to < 2 mm in the field, then stored at 4 °C. Globally, three samples for each area were analysed for their physical and chemical properties or characteristics. For the microbial analysis, all the samples collected were kept in sterile polythene bags at 4 °C.

Determination of physical and chemical properties
For each soil sample, humidity and texture were determined, by gravimetric method (ISO 11465: 1993), by pipette method (Gee & Bauder, 1986); respectively. pH was detected in H_2O (ISO 10390:2005), TOC % by using the Walkley Black Method (Ministero Politiche Agricole, 1999), and total N % by Kjeldhal Method (ISO 11261:1995).

Isolation and identification of soil fungi
Soils were homogenized in 0.8% NaCl sterile solution (1:5 w/v) (wet weigh basis); the suspended samples were incubated for 60 minutes at 25 °C under orbital agitation (90 rpm). Then 1 ml of diluted soil suspension (three replicates) was plated onto Malt Extract Agar (Oxoid) supplemented with Rose Bengal (80 ppm) and chloramphenicol (100 mg/l). The plates, incubated at 27 °C for 3-6 days, were observed daily. The colonies were counted and individually transferred to Malt Extract Agar. The identification of fungi at genera level was made on the basis of colony morphologies and of microscopic analysis.

Statistical analysis
The differences in chemical and physical characteristics of the two study areas were tested for significance by analysis of variance (ANOVA) techniques and by means of a post-hoc comparison test (Tukey's test) at $\alpha \leq 0.05$. The correlations between the frequency of fungi genera with soil parameters and between fungal genera were evaluated using Pearson's correlation coefficients. The software IBM SPSS Statistics 19(Chicago, Il, USA) was used.

Results and discussion

Physical and chemical characteristics
As reported in Table 1, the parameters show a high variability, mainly for TOC and total N content, but low pH variability.

Table 1. Physical and chemical properties (or characteristics) of the soils sampled in the two areas under study (g/100 g dry soil). The mean values, standard deviations and coefficients of variation (CV %) are reported (n = 9). Within each row the different letters mean that differences are significant according to post-hoc comparison test (Turkey's test) at $\alpha \leq 0.05$.

	Sardinia	CV %	Tunisia	CV %
Humidity %	27.14 a ±6.09	22	24.59 a ± 5.71	23
pH	6.01 a ± 0.54	9	6.67 b± 0.45	7
TOC %	7.16 a ± 1.53	21	5.06 b ± 1.28	25
Total N %	0.34 a ± 0.08	24	0.29 a ± 0.12	41
Sand %	72.05 a ± 3.42	5	59.88 b ± 3.28	5
Silt %	8.43 a ± 1.28	15	16.90 b± 1.45	9
Clay %	19.52 a ± 2.17	11	23.21 a ± 2.74	12

The same range of variability observed in Table 1 is found in pH, TOC and N from the soil of the mixed *Q. suber* forests of southern Spain by Aponte *et al.* (2011) who related them to the spatial heterogeneity of chemical elements in soil as well as to the "footprint" effect of plant species. The soil water content is mainly related to the sampling season (end of winter) and varies between 17 and 38% for Sardinia and between 17 and 34% for Tunisia soil. The humidity average value is slightly higher in Sardinia samples, even if the difference is not significant. The pH values are ranging from 5.19 to 6.81 and from 6.22 to 7.38, respectively for Sardinia and Tunisia soils. As for pH mean value, the soils of the two areas are significantly different (Table 1) and following the USDA classification (Soil Quality Indicators: pH 1998) they can be classified as moderately acid for Sardinia and neutral for Tunisia. According to literature data, soil pH is critical for cork oak growth: generally a pH in the range of 4.7 to 6.5 is considered favourable, and pH 7.8 has rarely been documented (Serrasolses *et al*., 2009). Both the study areas show pH values suitable for *Quercus suber* growth. Organic Carbon is recognized as one of the key chemical parameters of soil quality, and most forest soils contain between 0.3 and 11.5% in 20 cm depth (Lal, 2005). The soil samples from the two areas show a TOC ranging from 4 to 9% for Sardinia forest and from 3 to 6% for Tunisia forest, with a significant difference between the mean values thus falling in the literature range. The high variability of the detected values can be explained, considering that an amount of natural factors (precipitations, soil texture, etc.) can affect organic matter content in forest soil (Lal, 2005). According to the classification proposed by the Regional Agency of Veneto (ARPAV, 2007), both areas can be considered as rich in organic matter, as natural non managed environments. Total N in soils is ranging from 0.25 to 0.39% and from 0.20 to 0.43%, respectively for Sardinia and for Tunisia; with nosignificantly statistical differences between the two countries (Table 1).). Texture, climate and forest management can affect N content; moreover naturally invading N-fixing vegetation increase C and N in soil (Johnson & Curtis, 2001). The different vegetation of the two areas could partially explain the observed differences in TOC and total N. The sand content of the analyzed soils is in the range from 69 to 76% and from 56 to 63%, respectively for Sardinia and Tunisia, and the difference is statistically significant (Table 1) The clay content is from 16 to 21% for Sardinia and from 21 to 26% for Tunisia, but this difference is not statistically significant (Table 1). Following the USDA classification system (USDA, 1997), the texture of Sardinia soils is mainly classified as "sandy-loam" and of Tunisia as "sandy-clay-loam". According to studies on cork oak related to soil features, the most abundant soil texture where this species occurs is loamy and sandy (Serrasolses *et al.*, 2009). From this point of view, Sardinia area is suitable for *Q. suber*, while the more compact soil of Tunisia-Site (more clay and less sand) could create less favourable conditions for this species.

Fungi community

In Sardinia soil 16 fungal genera were found (Figure 1). The most frequent is *Trichoderma* (18%), followed by *Penicillium* (15%), *Paecilomyces* (13%) and *Cladosporium* (8%). The frequency of occurrence of the other genera range from 5 to 3%. In Tunisia 5 genera are identified (Figure 2). *Penicillium* (54%) is the dominant genus followed by *Aspergillus* (20%). The other genera: *Cladosporium*, *Fusarium* and *Rhizopus* are recorded with lower frequency (7%, 3% and 1%, respectively). *Penicillium*, *Aspergillus* and *Cladosporium* are recovered in both soils even if with different frequency of occurrence; *Fusarium* and *Rhizopus* are detected in Tunisia soil only, and *Trichoderma, Paecilomyces, Alternaria, Epicoccum, Mucor, Mortierella, Apiosordaria, Davidiella Neossartorya, Nigrospora, Talaromyces, Thermoascus and Umbelopsis* in Sardinia soil only.

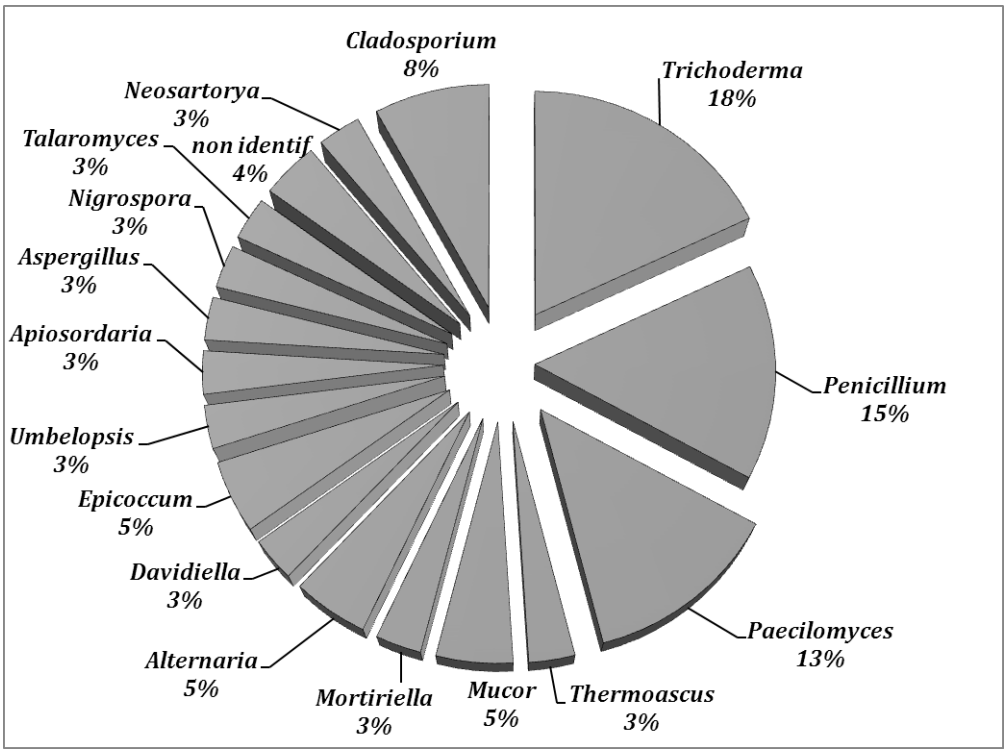

Figure 1. Fungi genera, average frequency of occurrence (%) in Sardinia soil.

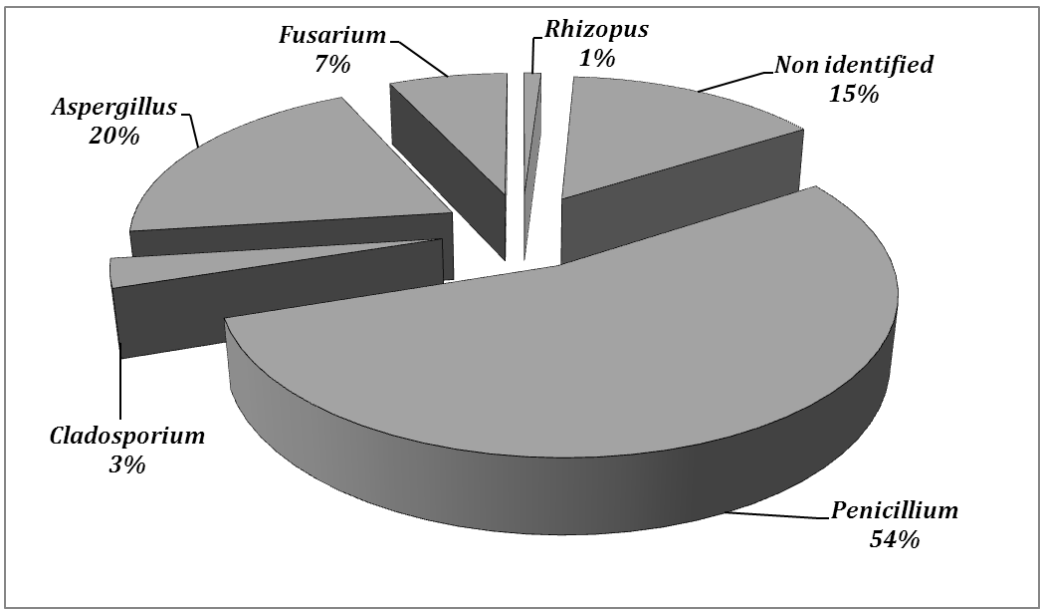

Figure 2. Fungi genera, average frequency of occurrence (%) in Tunisia soil.

In Table 2 are reported the correlations between the frequency of fungi genera/soil parameters and of fungi genera/fungi genera respectively. All the genera present in both Sardinia and Tunisia soils are considered, as also the genera present in one soil only but with a frequency ≥ 7%; the soil parameters showing significant correlation with at least one fungi genus are also reported.

Table 2. Pearson's correlation (r) of fungi genera frequency vs pedological parameters.

	TOC	Sand	Clay
Trichoderma	0.707	0.929**	-0.644
Penicillium	-0.641	-0.894**	0.705
Paecylomyces	0.720	0.935**	-0.632
Cladosporium	0.868*	0.994**	-0.414
Aspergillus	-0.598	-0.869*	0.742*
Fusarium	-0.586	-0.854*	0.760*

Significance level: **($\alpha \leq 0.01$); *($\alpha \leq 0.05$)

As shown in Table 2 sand is the only soil parameter that affects significantly the occurrence of all the fungi: its influence is positive for *Trichoderma*, *Paecilomyces* and *Cladosporium* and negative for the other genera.

The Pearson's correlation coefficient (r) of fungi genera versus fungi genera shows a significant negative influence of *Trichoderma* vs *Penicillium* (r = -0.995), *Aspergillus* (r = -0.989) and *Fusarium (r = -0.985)* occurrence, and of *Paecilomyces* vs *Penicillium (r = -0.993), Fusarium (r = -0.983)* and *Aspergillus (r = -0.986).* Moreover the presence of *Cladosporium* affects negatively *Penicillium (r = -0.937) Aspergillus (r = -0.917) and Fusarium (r = -0.902)* occurrence. While we observed a positive influence of *Trichoderma* on *Paecilomyces (r = 1)* and *Cladosporium* (r = 0.963) occurrence, of *Paecilomyces* on *Cladosporium (r = 0.966),* of *Aspergillus* on *Fusarium (r = 0.993)* and *Penicillium (r = 0.999)* and of *Penicillium* to *Fusarium (0.991).*

The main differences detected in this study are a lower number of genera, a higher frequency of *Penicillium* and *Aspergillus* and the presence of *Fusarium* and *Rhizopus* in Tunisia fungi community, compared to Sardinia fungi community. These differences can be related, to tree species and density, shrubs and overlaying vegetation, to the pedologic characteristics of the forest and to the fungi interactions. Many studies highlight clear correspondence between microfungal community composition and vegetation types. De Bellis *et al.* (2007) pointed out that in a mixed vegetation site different plant species may selectively stimulate some fungal species in the surrounding soil. Kara & Asan (2007) detected a different composition of fungi community in conifer and hardwood forests. The authors suggested, as Kuiters (1999) and Phriha *et al*. (2001) that this is due to the pine litter leaching of substances recalcitrant to fungi decomposition and/or inhibitory for some fungi present in the surface soil. Moreover studies reported that *Penicillium* and *Aspergillus* species are able to metabolize complex carbon compounds and the phenolic compounds present in the litter better than other species (Kjoller & Struwe, 2005). The presence of pine trees in the Tunisian

forest could partially explain the low number of genera isolated in this forest compared to the genera isolated in Sardinia and the high frequency of *Penicillium* and *Aspergillus* in Tunisia soil. The highest frequencies of *Aspergillus* and *Penicillium* in Tunisia forest could also be related to the clay content according to Kara & Bolat (2007). In addition, the metabolic interaction between the fungi can influence the fungal community of the two soils. Some works report that *Trichoderma* is a suppressor of *Fusarium, Cladosporium, Penicillium and Aspergillus* (Lone *et al.*, 2012) and that *Cladosporium* has an antagonistic activity on *Penicillium, Aspergillus* and *Fusarium* (Chalfoun, 2010). As reported in literature, several mechanisms including nutrient subtraction, production of volatile and nonvolatile toxins, extracellular enzymes and hyphae interferences occur in fungal competitions (Chalfoun, 2010). Moreover fungal species may interact mutualistically i.e. each facilitates the success of the other (Rayner & Webber, 1984).

Acknowledgements

This research was part funded by NATO, Science for Peace, Porject ESP.MD.SFPP981674. With thanks to Sylvo-Pastoral Institute of Tabarka, Tunisia, and AGRIS Sardegna , Italy, for granting permission to sample.

References

Aponte, C., Garcıa, L. V., Perez-Ramos, I. M., Gutierrez, E. & Marañón, T. 2011: Oak trees and soil interactions in Mediterranean forests: a positive feedback model. J. Vegetation Science 22: 856-867.

ARPAV 2007: L'interpretazione delle analisi del terreno – Strumento per la sostenibilità ambientale. www.apra.veneto.it

Barberis, A., Dettori, S. & Filigheddu, M. R. 2003: Management problems in mediterranean cork oak forests: post-fire recovery. J. Arid Environm. 54: 565-569.

Chalfoun, S. M. 2010: Biological control and bioactive microbial metabolites: a coffee quality perspective. Ciênc. Agrotec. Lavras 34: 1071-1085.

Christensen, M. 1981: Species diversity and dominance in fungal community. In: The fungal community, its organization and role in the ecosystem (eds. Wicklow, D. T. & Carroll, G. C.): 201-232. Marcel Dekker Inc.

Croitoru, L. 2007: Valuing the non-timber forest products in the Mediterranean region. Ecol. Econ. 63: 768-775.

De Bellis, T., Kernaghan, G. & Widden, P. 2007: Plant community influences on soil microfungal assemblages in boreal mixed-wood forests. Mycologia 99: 356-367.

Gee, G. W. & Bauder, J. W. 1986: Particle-size Analysis. In: Methods of soil analysis, Part 1, Physical and mineralogical methods. Second Edition (ed. Klute, A.): 383-411. Agronomy.

Johnson, D. W. & Curtis, P. S. 2001: Effects of forest management on C and N storage: meta analysis. Forest Ecol. Manag. 140: 227-238.

Kara, O. & Asan, A. 2007: Microfungal community structure from forest soil in Northern Thrace Region, Turkey. Ann. Microbiol. 57: 149-155.

Kara, O. & Bolat, I. 2007: Influence of soil compaction on microfungal community structure in two soil types in Bartin Province. Turkey. J. Basic Mircobiol. 47: 394-399.

Kjoller, A. H. & Struwe, S. 2005: Fungal communities, succession, enzymes, and decomposition. In: Enzymes in the environment: Activity, ecology and applications (eds. Burns, R. G., Dick, R. P.): 305-323. Marcel Dekker, New York.

Kuiters, A. T. 1999: Role of phenolic substances from decomposing forest litter in plant-soil interaction. Acta Bot. Neer. 39: 329-348.

Lal, R. 2005: Forest soils and carbon sequestration. Forest Ecol. Manag. 220: 242-258.

Lone, M. A., Wani, M. R., Sheikh, S. A., Sahay, S. & Dar, M. S. 2012: Antagonistic potentiality of *Trichoderma harzianum* against *Cladosporium spherospermum*, *Aspergillus niger* and *Fusarium oxysporum*. J. Biol. Agricult. Healthcare 2: 72-76.

Magri, D., Fineschi, S., Bellarosa, R., Buonamici, A., Sebastiani, F., Schirone, B., Simeone, M. C. & Vendramin, G. G. 2007: The distribution of *Quercus suber* chloroplast haplotypes matches the paleogeographical history of the western Mediterranean. Mol. Ecol 16: 5259-5266.

Ministero Politiche Agricole 1999: Metodi Ufficiali di Analisi chimica del Suolo. D.M. 13/07/99, G.U. Repubblica Italiana n. 248, 21/19/99.

Pereira, H. 2007: Cork, biology, production and uses. Elsevier publications, Amsterdam.

Pérez-Ramos, I. M., Zavala, M. A., Marañón, T., Díaz-Villa, M. D. & Valladares, F. 2008: Dynamics of understorey herbaceous plant diversity following shrub clearing of cork oak forests: a five-year study. Forest Ecol. Manag. 255: 3242-3253.

Phriha, O., Grayston, S. J., Hiukka, R., Pennanen, T. & Smolander, A. 2001: Microbial community structure and characteristics of the organic matter in soil under *Pinus sylvestris*, *Picea abies* and *Betula pendula* at two forest sites. Biol. Fert. Soils 33: 17-24.

Rayner, A. D. M. & Webber, J. F. 1984: Interspecific mycelial interactions: an overview. In: The Ecology and Physiology of the fungal mycelium (eds. Jennings, D. H. & Rayner, A. D.): 383-417. Cambridge University Press, Cambridge.

Serra, G., Tomasi, D., Khaldi, A., Nouri, M., Carta, L., Loddo, S., Camarda, I., Nejib Rejeb, M. & Aru, A. 2005: Conservation and recovering of cork oak forests. First results of cooperation between Sardinia (Italy) and Tunisia. IOBC-WPRS Bull. 28(8): 253-260.

Serrasolses, I., Perez-Devesa, M., Vilagrosa, A., Pausas, J. G., Sauras, T., Cortina, J. & Vallejo, V. R. 2009: Soil properties constraining cork oak distribution. In: Cork Oak Woodlands on the Edge (eds. Aronson, J. *et al*.): 89-102. Island Press.

Silva, J. S., Catry, F. 2006: Forest fires in cork oak (*Quercus suber* L.) stands in Portugal. Internat. J. Environm. Studies 63: 235-257.

USDA 1997: Soil Taxonomy: a basic System of Soil Classification for making and interpreting Soil Survey. U.S. Department of Agriculture Soil Conservation Service.